

Limitations of urease test in diagnosis of pediatric *Helicobacter pylori* infection

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Abstract

The diagnosis of *Helicobacter pylori* (*H. pylori*) infection is usually based on the results of urease test and histology. The urease test known as a simple and cheap method does not need special skills to perform or to read the result. The time needed for the test to turn positive depends on the concentration of bacteria, and the accuracy is up to the density of *H. pylori* density in the biopsy sample, which is generally lower in children than adolescents and adults. Therefore, there are debates about the sensitivity of the urease test in children. The reason for lower sensitivity of the urease test in children was not identified, but might be related to the low density and patchy distribution of bacteria. In this review, we discuss the limitations of the urease test in children according to age, histology, number of biopsy samples, and biopsy site. In children under 5 years old, the differences in positivity rate when the urease test used one or three biopsy samples, and samples from the antrum or the gastric body, were larger than those in children aged 5-15 years. Thus, three or more biopsy samples from both the antrum and body would improve the sensitivity of *H. pylori* infection diagnosis in children under 5 years old.

Key words: *Helicobacter pylori* infection; Urease test; Children

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Core tip: The diagnosis of *Helicobacter pylori* (*H. pylori*) infection is usually based on the findings of histology and the results of the urease test. However, the sensitivity of urease test in children was lower compared to adults. The lower sensitivity of urease test of children might be related to the low density and patchy distribution of bacteria. In urease test, three or more biopsy samples

from both the antrum and body would improve the sensitivity of *H. pylori* infection diagnosis in children under 5 years old.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is the main pathogen of acute and chronic gastritis, peptic ulcer disease, and gastric cancer^[1,2]. Although primary *H. pylori* infection occurs during early childhood, most adults eventually become *H. pylori* carriers in developing countries^[3,4]. In children, abdominal pain is the most common symptom in *H. pylori* infection^[5]. Whether or not *H. pylori*-infected children with recurrent abdominal pain should be treated did not reach consensus^[6]. However, a positive family history of gastric cancer is one of the risk factors of adulthood gastric cancer in children with *H. pylori* infection^[7]. Thus, a reliable diagnostic test to detect *H. pylori* infection in children is required. The most reliable diagnostic method of *H. pylori* infection in children is endoscopy with gastric biopsy^[8].

The diagnosis of *H. pylori* infection is usually based on the findings of histology and the results of the urease test^[9]. Histology plays an important role in detecting *H. pylori* and also indicates the degree of chronic and active inflammation in the stomach^[10]. The urease test known as a simple and cheap method does not need special skills to perform or to read the result. The urease test uses phenol red, which changes from yellow to pink or red as the pH increases. *H. pylori* can stay alive in the human stomach and activate its own cytoplasmic urease, converting urea into carbon dioxide and ammonia and increasing the pH of the surrounding environment^[11]. Buffered urease tests need at least 10^5 organisms so as to produce a positive result^[12]. Therefore, the urease test may be negative, if biopsy samples in which the histology reveals only one or two bacteria in the entire section^[13]. The time needed for the test to show a positive result depends on the concentration of bacteria^[14], and the accuracy of the urease test primarily is up to the density of *H. pylori* in the gastric sample. Generally the *H. pylori* density is found to be lower in children than adolescents and adults^[15]. The time of the urease test turns positive is up to the concentration of bacteria and the temperature. Most commercially available urease tests will turn positive within 2-3 h but it is best to hold those that appear negative for 24 h regardless of age^[14]. The urease enzyme activity of *H. pylori* at the diagnosis also affects the urease test. Acid suppression by proton pump inhibitor is a well-known cause of the false negative result of urease test^[14].

However, there is currently a debate about the sensitivity of the urease test in children without consideration of the urease enzyme activities. A review of the literature about *H. pylori* diagnostic tests in children from 1999-2009 suggested that prompt urease tests have better sensitivity than histology to detect the occurrence of *H. pylori*^[16]. In 1590 pediatric patients from 1989 to 2009, urease tests had lower sensitivity (83.4%) and comparable specificity (99.0%) with histology^[17]. The reason for this lower sensitivity in children was not identified, but it might be related to the low density and patchy distribution of bacteria^[15,18]. In this review, we discuss the limitations of the urease test in children based on the literature reporting on pediatric patients.

AGE OF CHILDREN

Age is another factor influencing the sensitivity of the urease test^[17,19,20]. The age ranges of the children in different studies were wide, and varied from ≤ 15 to ≤ 18 years of age^[5-8]. In children, a low density of *H. pylori* has been reported^[15,21], which can be attributable to sampling errors such as very low numbers of *H. pylori* in the tissue samples or patchy distribution of the organism in the stomach's mucosa^[22]. Among 530 children infected with *H. pylori*, the urease test was positive in 442, and the rate of urease tests was positively associated with the children aged 5 years old and above compared with those below 5 years old^[17]. The false negative rate for the urease test was 16.6% in these children, mostly found in those below 5 years old^[17]. In our previous studies, we divided pediatric patients into three age groups: 0-4 years, 5-9 years, and 10-15 years^[19,20]. The positivity rate of the urease test increased with each increasing age group, with the lowest rate in the 0-4 years group and highest in the 10-15 years group (Figure 1)^[20]. We then compared the time points at which the positive reaction produced, dividing these into 0-1 h, 1-6 h, 6-24 h, and 24-48 h. This was considered within each of 4 age groups: 0-4 years, 5-9 years, 10-14 years, and 20-29 years^[20]. Positive results occurred within 1 h in the older age groups, 10-15 years and 20-29 years. Conversely, most positive results occurred at 6-24 h in the 0-4 years group using body biopsy specimens (Figure 1)^[20]. This result suggested that a low degree of *H. pylori* colonization in gastric biopsy samples might be more frequent in children under 5 years old.

HISTOPATHOLOGICAL GRADES AND THE POSITIVITY RATE OF THE UREASE TEST

H. pylori infection causes chronic inflammation in stomach mucosa. Histology has been regarded as the golden compass for *H. pylori* detection^[9], as it can detect the bacteria as well as the severity of inflammation. However, just as a positive urease test requires approximately 10^5 *H. pylori* in the biopsy sample^[12],

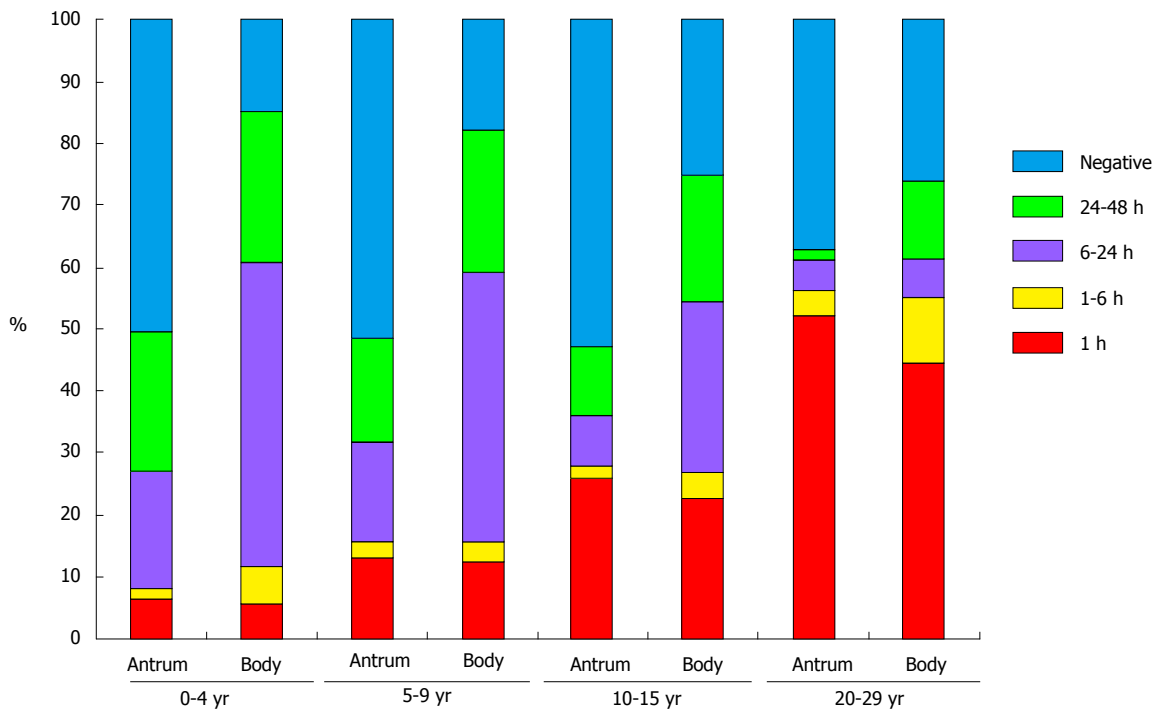


Figure 1 The positivity rate and positive timing of the urease test both in the antrum and body according to age. The positivity rate of the urease test in the antrum was higher in 20-29 years group than that in other three age groups, and the positivity rate of the urease test in the body decreased with increasing age ($P < 0.0001$). The highest positivity timing was within 1 h in the 20-29 years group, and within 6-24 h in children ($P < 0.0001$)^[20].

Table 1 The studies about the positivity rate of urease test by comparing to biopsy sites and biopsy numbers

Ref.	Published year	No. subjects	Mean age (yr)	Comparison	Positivity rate of urease test (%)
Siddique <i>et al</i> ^[24]	2008	100 adults	36.1	One biopsy	52
				Two biopsies	68
				Three biopsies	76
				Four biopsies	96
Seo <i>et al</i> ^[19]	2014	255 children	NA	One biopsy	32.2
				Three biopsies	40.1
Moon <i>et al</i> ^[25]	2012	214 adults	53.6	Antrum	58.9
				body	62.1
				Antrum + body	69.2
Lan <i>et al</i> ^[26]	2012	164 adults	NA	Antrum	83.3
				Antrum + corpus	100

NA: Not available.

histopathological results are limited by the distribution and density of the bacteria in the sample. Therefore, the patchy distribution and low density of *H. pylori* in gastric mucosa in children might be related to the absence of bacteria in some histopathological findings. Nevertheless, the positive urease test is highly correlated with the density of bacteria, the severity of chronic gastritis, and the presence of active gastritis, as determined by histopathology^[15,17].

When histopathological evaluations found severe degrees of chronic and active gastritis, or *H. pylori* infiltration, the urease tests revealed a quick change of color, indicating positive results in samples from both the antrum and gastric body, regardless of age, compared with those from milder histopathological grades^[20]. In 38 children, aged 2-18 years, the accuracy

of the rapid urease test was 95% for samples determined to be positive by histology^[23]. The accuracy of the urease test is up to the density of *H. pylori* in the gastric sample. However the *H. pylori* density is overall lower in children than in adolescents and adults^[15]. Therefore, both histology and a urease test should be performed in children for diagnosis of *H. pylori* infection.

EFFECT OF THE NUMBER OF GASTRIC BIOPSY SPECIMENS

Two or more numbers of gastric biopsies improve the sensitivity and saves the time for the positive result for diagnosis of urease test^[19,24]. There were several studies comparing positivity rate of urease test according to the biopsy numbers diagnosis (Table 1): three were

adults studies and one was children study. In children, the positivity rate of the urease test was higher in three biopsy samples than single biopsy sample, in the same child^[19]. The discrepancy between positivity rates of the urease test utilizing one vs three biopsy samples was elevated in children below 5 years old^[21]. Thus, when the urease test is performed on three or more biopsy samples, it is possibly a more accurate diagnostic modality.

In adults, the positivity rate of urease test also increased with using two to four biopsy specimens than a single biopsy specimen^[24]. The discrepancy rates of one and two or more biopsy specimens were higher in adults (10.3%, 16.7% and 16%) than in children (7.9%) (Table 1)^[19,24-26]. In adults, the time for positive urease test was faster in united test (1.69 h for antrum and body) than in separate test (3.58 h for body or antrum) on average^[25]. In children, it is reported to be a low degree of *H. pylori* colonization and more patchy distribution of *H. pylori*^[21]. Thus, when the urease test is performed on three or more biopsy samples, it may be a more accurate diagnostic modality.

EFFECT OF BIOPSY SITE ON UREASE TEST RESULTS

Most patients were taken of biopsy specimens from the antrum, as one biopsy from the gastric angle for urease test had the maximum probability for detecting *H. pylori* infection^[27]. The use of an additional biopsy from the gastric body can improve the detection rate of *H. pylori* (Table 1)^[20,25,26]. In our previous study^[20], the discrepancy of urease test results was bigger between the antrum and body in children compared with that in adults, and the most difference was found in the samples that showed a positive color change late in testing, from 6-24 h (Figure 1). In our study, no difference was observed between the speed of a positive reaction in samples from the antrum and from the body in adults^[20]. But the time of positive test was 3.58 h for the separate test and 1.69 h for the united test on average^[25]. The colonization degree was remarkably lower in the body compared with the antrum^[21]. In our previous study, the histopathologic findings of the antrum and body revealed similar degrees of chronic and active gastritis and infiltration of bacteria in children, the positivity rate of the urease test was higher in samples from the body than from the antrum^[20]. This result was similar to the study of Korean adults, where the positivity rate of urease test in body was higher than in antrum (Table 1)^[25]. In adults, the sensitivity of urease test decreased along with the degree of gastritis with atrophy increasing from 100% in normal, 97% in mild, 91% in moderate to 66% in severe^[26]. However additional corpus biopsy resulted in increased sensitivity to 16.67% compared to single antrum biopsy (Table 1)^[26]. This might also be related to the patchy distribution of *H. pylori* and low density of the organism in body. There has been a

continuing debate about the optimum site and number of gastric biopsies for the diagnosis of *H. pylori*.

CONCLUSION

Early diagnosis of *H. pylori* infection is crucial for symptomatic children with a family history of gastric cancer. In children, endoscopic examination and biopsy for histology and urease test is still the most reliable method for diagnosis of *H. pylori* infection. The difference in the positivity rate of the urease test between using one or three biopsy samples, or using biopsy samples from the antrum or body, were larger in children aged 0-5 years than 5-15 years. Use of three or more biopsy samples from both the antrum and body would improve the sensitivity of *H. pylori* detection in children.

REFERENCES

- 1 **Queiroz DM**, Carneiro JG, Braga-Neto MB, Fialho AB, Fialho AM, Goncalves MH, Rocha GA, Rocha AM, Braga LL. Natural history of *Helicobacter pylori* infection in childhood: eight-year follow-up cohort study in an urban community in northeast of Brazil. *Helicobacter* 2012; **17**: 23-29 [PMID: 22221612 DOI: 10.1111/j.1523-5378.2011.00894.x]
- 2 **Shiotani A**, Cen P, Graham DY. Eradication of gastric cancer is now both possible and practical. *Semin Cancer Biol* 2013; **23**: 492-501 [PMID: 23876852 DOI: 10.1016/j.semcancer.2013.07.004]
- 3 **Malaty HM**, El-Kasabany A, Graham DY, Miller CC, Reddy SG, Srinivasan SR, Yamaoka Y, Berenson GS. Age at acquisition of *Helicobacter pylori* infection: a follow-up study from infancy to adulthood. *Lancet* 2002; **359**: 931-935 [PMID: 11918912 DOI: 10.1016/S0140-6736(02)08025-X]
- 4 **Rhee KH**, Youn HS, Baik SC, Lee WK, Cho MJ, Choi HJ, Maeng KY, Ko KW. Prevalence of *Helicobacter pylori* infection in Korea. *J Korean Soc Microbiol* 1990; **25**: 475-490
- 5 **Koletzko S**, Jones NL, Goodman KJ, Gold B, Rowland M, Cadranet S, Chong S, Colletti RB, Casswall T, Elitsur Y, Guarner J, Kalach N, Madrazo A, Megraud F, Oderda G. Evidence-based guidelines from ESPGHAN and NASPGHAN for *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2011; **53**: 230-243 [PMID: 21558964 DOI: 10.1097/MPG.0b013e3182227e90]
- 6 **Ozçay F**, Koçak N, Temizel IN, Demir H, Ozen H, Yüce A, Gürakan F. *Helicobacter pylori* infection in Turkish children: comparison of diagnostic tests, evaluation of eradication rate, and changes in symptoms after eradication. *Helicobacter* 2004; **9**: 242-248 [PMID: 15165260 DOI: 10.1111/j.1083-4389.2004.00230.x]
- 7 **Cam S**. Risk of gastric cancer in children with *Helicobacter pylori* infection. *Asian Pac J Cancer Prev* 2014; **15**: 9905-9908 [PMID: 25520126 DOI: 10.7314/APJCP.2014.15.22.9905]
- 8 **Gold BD**, Colletti RB, Abbott M, Czinn SJ, Elitsur Y, Hassall E, Macarthur C, Snyder J, Sherman PM. *Helicobacter pylori* infection in children: recommendations for diagnosis and treatment. *J Pediatr Gastroenterol Nutr* 2000; **31**: 490-497 [PMID: 11144432 DOI: 10.1097/00005176-200011000-00007]
- 9 **Wong A**, Ching SS, Long AS. The use of a second biopsy from the gastric body for the detection of *Helicobacter pylori* using rapid urease test. *Singapore Med J* 2014; **55**: 644-647 [PMID: 25630318 DOI: 10.11622/smedj.2014178]
- 10 **Aydin O**, Egilmez R, Karabacak T, Kanik A. Interobserver variation in histopathological assessment of *Helicobacter pylori* gastritis. *World J Gastroenterol* 2003; **9**: 2232-2235 [PMID: 14562384]
- 11 **Berger A**. Scientists discover how *helicobacter* survives gastric acid. *BMJ* 2000; **320**: 268 [PMID: 10650011 DOI: 10.1136/bmj.320.7230.268]
- 12 **Mégraud F**, Bessède E, Lehours P. Current methods used for the diagnosis of *Helicobacter pylori* infection. In: Buzás GM.

- Helicobacter pylori* - A Worldwide Perspective 2014. Oak Park: Bentham Science, 2014: 234-258 [DOI: 10.2174/9781608057375114010014]
- 13 **Ohkusa T**, Miwa H, Endo S, Okayasu I, Sato N. *Helicobacter pylori* is a fragile bacteria when stored at low and ultra-low temperatures. *J Gastroenterol Hepatol* 2004; **19**: 200-204 [PMID: 14731131 DOI: 10.1111/j.1440-1746.2004.03266.x]
 - 14 **Uotani T**, Graham DY. Diagnosis of *Helicobacter pylori* using the rapid urease test. *Ann Transl Med* 2015; **3**: 9 [PMID: 25705641 DOI: 10.3978/j.issn.2305-5839.2014.12.04]
 - 15 **Elitsur Y**, Hill I, Lichtman SN, Rosenberg AJ. Prospective comparison of rapid urease tests (PyloriTek, CLO test) for the diagnosis of *Helicobacter pylori* infection in symptomatic children: a pediatric multicenter study. *Am J Gastroenterol* 1998; **93**: 217-219 [PMID: 9468245 DOI: 10.1111/j.1572-0241.1998.00217.x]
 - 16 **Guarner J**, Kalach N, Elitsur Y, Koletzko S. *Helicobacter pylori* diagnostic tests in children: review of the literature from 1999 to 2009. *Eur J Pediatr* 2010; **169**: 15-25 [PMID: 19618211 DOI: 10.1007/s00431-009-1033-x]
 - 17 **Roma-Giannikou E**, Roubani A, Sgouras DN, Panayiotou J, van-Vliet C, Polyzos A, Roka K, Daikos G. Endoscopic tests for the diagnosis of *Helicobacter pylori* infection in children: Validation of rapid urease test. *Helicobacter* 2010; **15**: 227-232 [PMID: 20557365 DOI: 10.1111/j.1523-5378.2010.00756.x]
 - 18 **Midolo P**, Marshall BJ. Accurate diagnosis of *Helicobacter pylori*. Urease tests. *Gastroenterol Clin North Am* 2000; **29**: 871-878 [PMID: 11190071]
 - 19 **Seo JH**, Park JS, Yeom JS, Lim JY, Park CH, Woo HO, Baik SC, Lee WK, Cho MJ, Rhee KH, Youn HS. Correlation between positive rate and number of biopsy samples on urease test in childhood *Helicobacter pylori* infection. *J Korean Med Sci* 2014; **29**: 106-109 [PMID: 24431913 DOI: 10.3346/jkms.2014.29.1.106]
 - 20 **Seo JH**, Youn HS, Park JJ, Yeom JS, Park JS, Jun JS, Lim JY, Park CH, Woo HO, Ko GH, Baik SC, Lee WK, Cho MJ, Rhee KH. Influencing Factors to Results of the Urease Test: Age, Sampling Site, Histopathologic Findings, and Density of *Helicobacter pylori*. *Pediatr Gastroenterol Hepatol Nutr* 2013; **16**: 34-40 [PMID: 24010104 DOI: 10.5223/pghn.2013.16.1.34]
 - 21 **Drumm B**. *Helicobacter pylori* in the pediatric patient. *Gastroenterol Clin North Am* 1993; **22**: 169-182 [PMID: 8449565]
 - 22 **Carelli AP**, Patrício FR, Kawakami E. Carditis is related to *Helicobacter pylori* infection in dyspeptic children and adolescents. *Dig Liver Dis* 2007; **39**: 117-121 [PMID: 17196450 DOI: 10.1016/j.dld.2006.10.012]
 - 23 **Jolley CD**, Wagner DA. Comparison of the ¹³C-urea blood test to histology and rapid urease testing in the diagnosis of *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2007; **44**: 68-70 [PMID: 17204956 DOI: 10.1097/01.mpg.0000243426.78721.bc]
 - 24 **Siddique I**, Al-Mekhaizeem K, Alateeqi N, Memon A, Hasan F. Diagnosis of *Helicobacter pylori*: improving the sensitivity of CLOtest by increasing the number of gastric antral biopsies. *J Clin Gastroenterol* 2008; **42**: 356-360 [PMID: 18277905 DOI: 10.1097/MCG.0b013e31802b650d]
 - 25 **Moon SW**, Kim TH, Kim HS, Ju JH, Ahn YJ, Jang HJ, Shim SG, Kim HJ, Jung WT, Lee OJ. United Rapid Urease Test Is Superior than Separate Test in Detecting *Helicobacter pylori* at the Gastric Antrum and Body Specimens. *Clin Endosc* 2012; **45**: 392-396 [PMID: 23251887 DOI: 10.5946/ce.2012.45.4.392]
 - 26 **Lan HC**, Chen TS, Li AF, Chang FY, Lin HC. Additional corpus biopsy enhances the detection of *Helicobacter pylori* infection in a background of gastritis with atrophy. *BMC Gastroenterol* 2012; **12**: 182 [PMID: 23272897 DOI: 10.1186/1471-230X-12-182]
 - 27 **Woo JS**, el-Zimaity HM, Genta RM, Yousfi MM, Graham DY. The best gastric site for obtaining a positive rapid ureas test. *Helicobacter* 1996; **1**: 256-259 [PMID: 9398877]

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